New Phytologist Supporting Information

**Title:** Replaying the evolutionary tape to investigate subgenome dominance in allopolyploid *Brassica napus*

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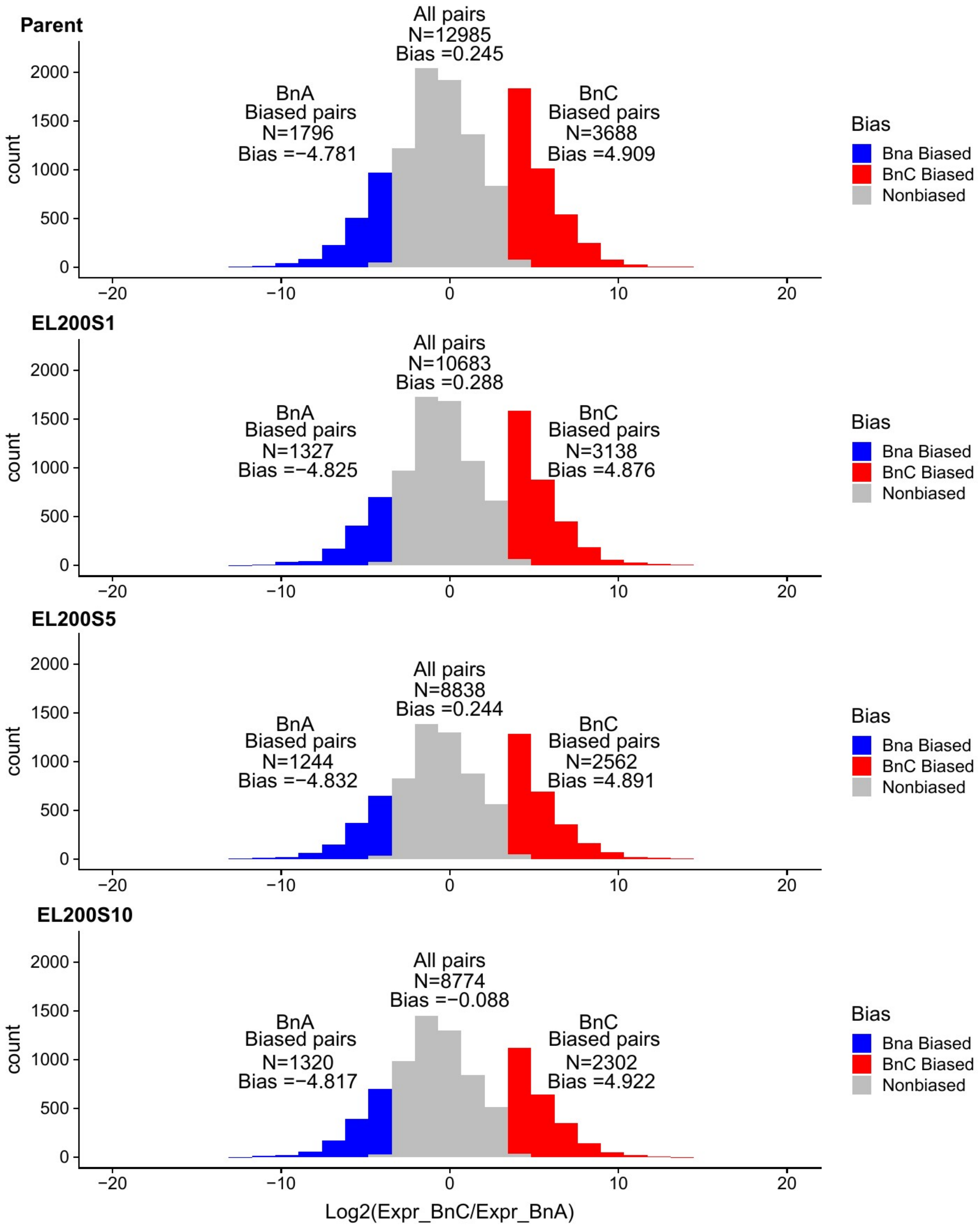
**Figure S1.**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Bias =0.245
BnA
Biased pairs
N=1796
Bias =−4.781
BnC
Biased pairs
N=3688
Bias =4.909
EL200S1
All pairs
N=10683
Bias =0.288
BnA
Biased pairs
N=1327
Bias =−4.825
BnC
Biased pairs
N=3138
Bias =4.876
EL200S5
All pairs
N=8838
Bias =0.244
BnA
Biased pairs
N=1244
Bias =−4.832
BnC
Biased pairs
N=2562
Bias =4.891
EL200S10
All pairs
N=8774
Bias =−0.088
BnA
Biased pairs
N=1320
Bias =−4.817
BnC
Biased pairs
N=2302
Bias =4.922
count
0
500
1000
1500
2000
−20
−10
0
10
20
Log2(Expr_BnC/Expr_BnA)
Bias
Bna Biased
BnC Biased
Nonbiased

**Figure S2**

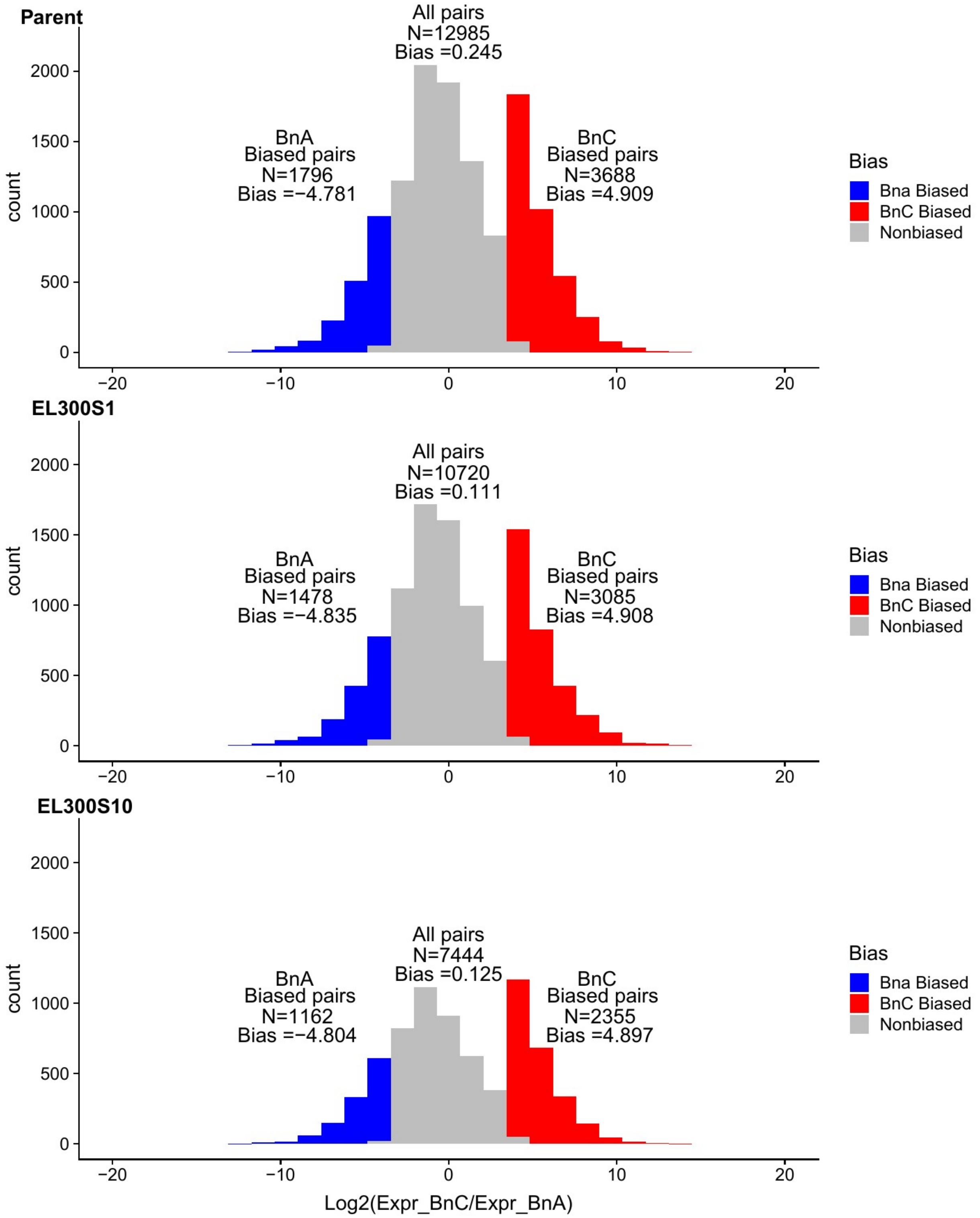

Parent
All pairs
N=12985
Bias =0.245
BnA
Biased pairs
N=1796
Bias =−4.781
BnC
Biased pairs
N=3688
Bias =4.909
EL300S1
All pairs
N=10720
Bias =0.111
BnA
Biased pairs
N=1478
Bias =−4.835
BnC
Biased pairs
N=3085
Bias =4.908
EL300S10
All pairs
N=7444
Bias =0.125
BnA
Biased pairs
N=1162
Bias =−4.804
BnC
Biased pairs
N=2355
Bias =4.897
count
0
500
1000
1500
2000
−20
−10
0
10
20
Log2(Expr_BnC/Expr_BnA)
Bias
Bna Biased
BnC Biased
Nonbiased

**Figure S3.**

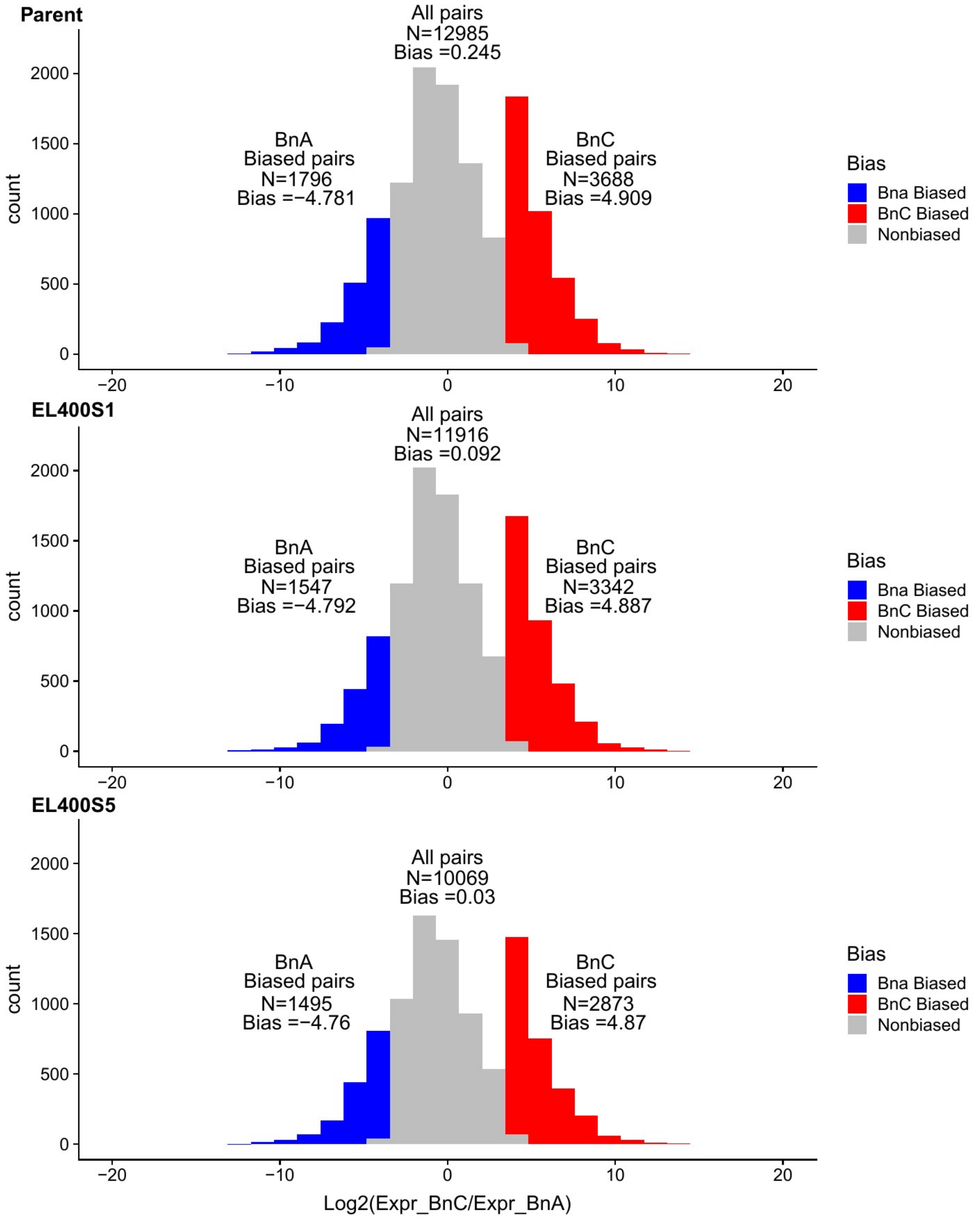

Parent
All pairs
N=12985
Bias =0.245
BnA
Biased pairs
N=1796
Bias =−4.781
BnC
Biased pairs
N=3688
Bias =4.909
count
2000
1500
1000
500
0
−20
−10
0
10
20
Bias
Bna Biased
BnC Biased
Nonbiased
EL400S1
All pairs
N=11916
Bias =0.092
BnA
Biased pairs
N=1547
Bias =−4.792
BnC
Biased pairs
N=3342
Bias =4.887
count
2000
1500
1000
500
0
−20
−10
0
10
20
Bias
Bna Biased
BnC Biased
Nonbiased
EL400S5
All pairs
N=10069
Bias =0.03
BnA
Biased pairs
N=1495
Bias =−4.76
BnC
Biased pairs
N=2873
Bias =4.87
count
2000
1500
1000
500
0
−20
−10
0
10
20
Log2(Expr_BnC/Expr_BnA)
Bias
Bna Biased
BnC Biased
Nonbiased

**Figure S4.**

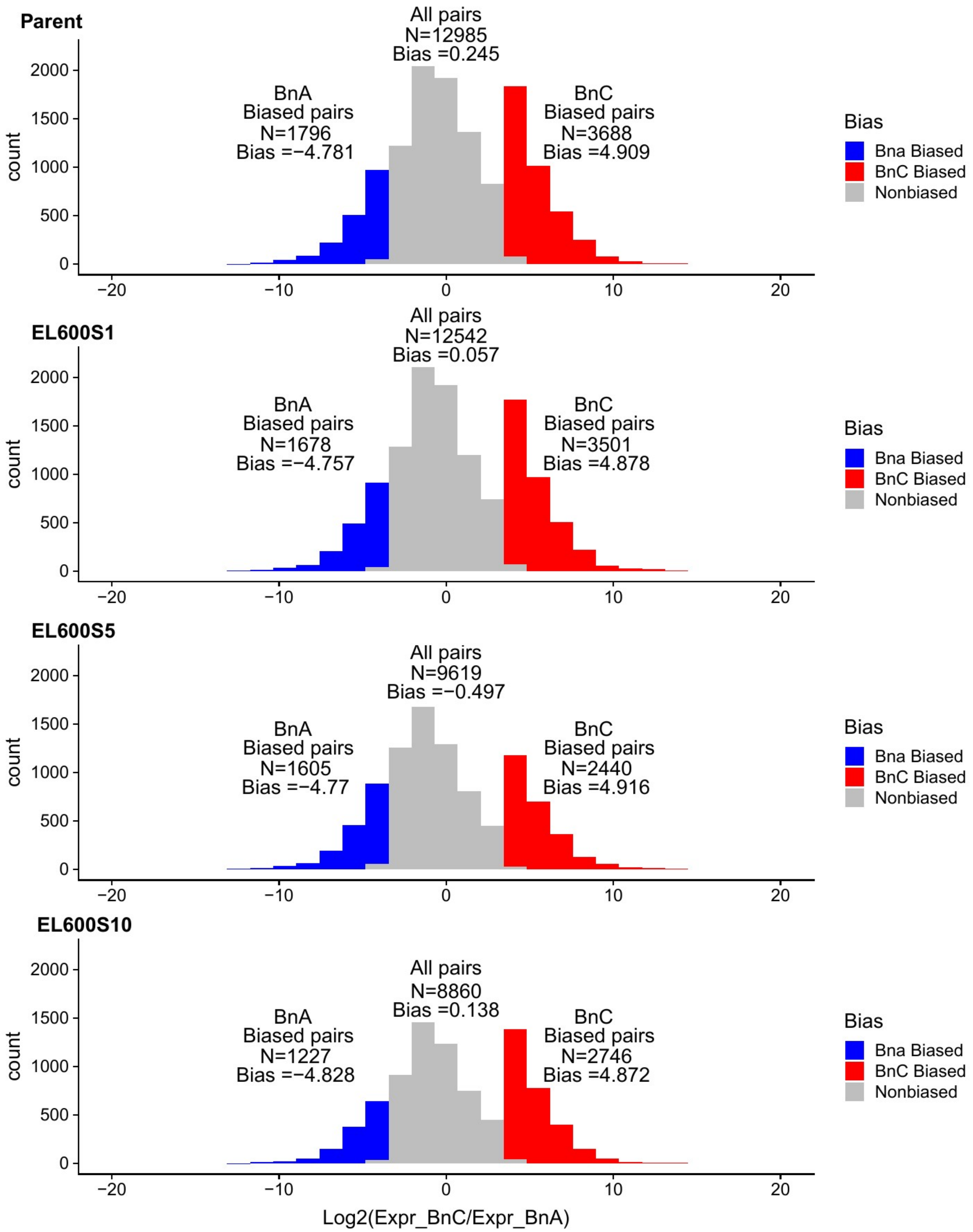
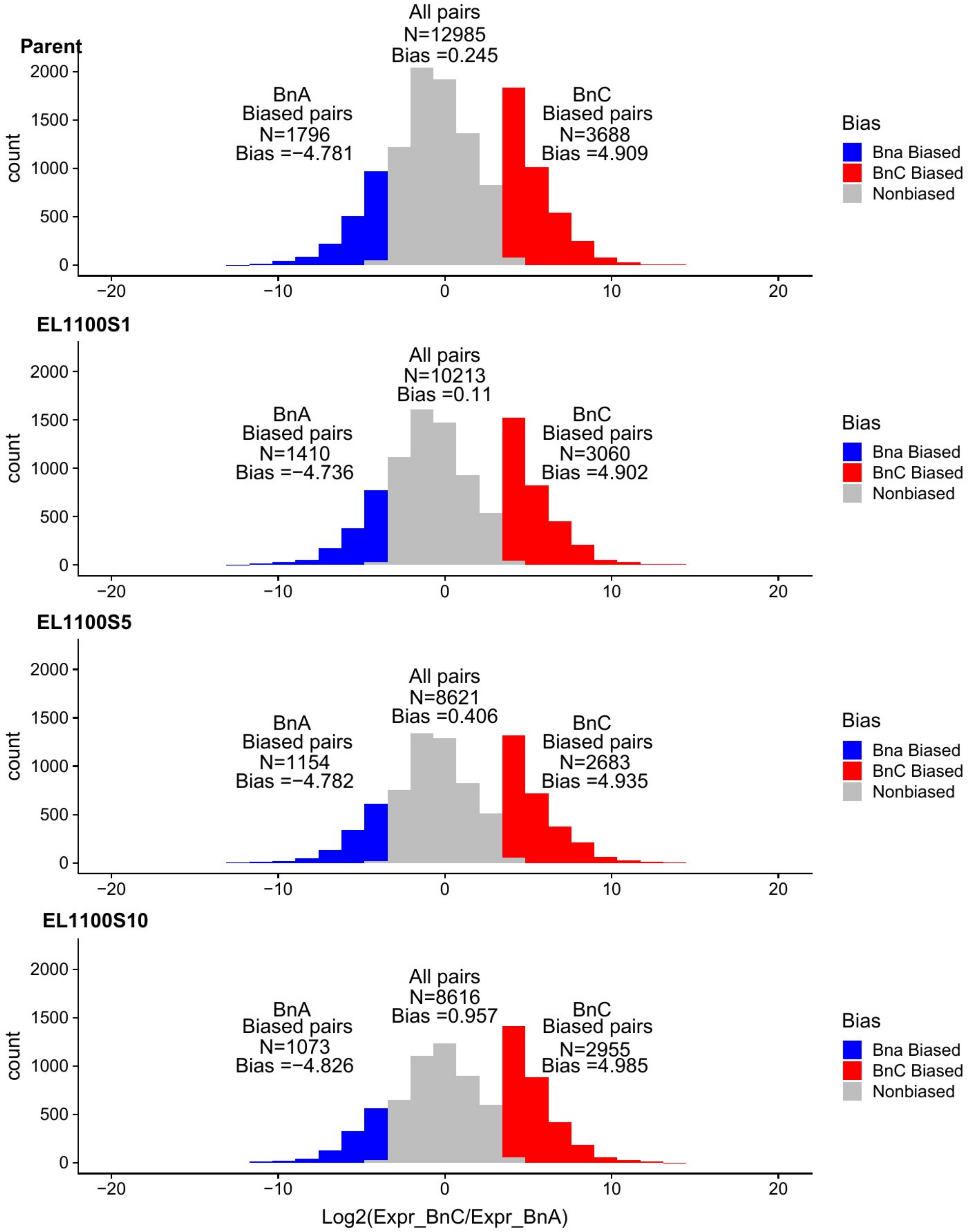

Parent
All pairs
N=12985
Bias =0.245
BnA
Biased pairs
N=1796
Bias =−4.781
BnC
Biased pairs
N=3688
Bias =4.909
count
0
500
1000
1500
2000
−20
−10
0
10
20
Bias
Bna Biased
BnC Biased
Nonbiased
EL600S1
All pairs
N=12542
Bias =0.057
BnA
Biased pairs
N=1678
Bias =−4.757
BnC
Biased pairs
N=3501
Bias =4.878
count
Bias
Bna Biased
BnC Biased
Nonbiased
EL600S5
All pairs
N=9619
Bias =−0.497
BnA
Biased pairs
N=1605
Bias =−4.77
BnC
Biased pairs
N=2440
Bias =4.916
count
Bias
Bna Biased
BnC Biased
Nonbiased
EL600S10
All pairs
N=8860
Bias =0.138
BnA
Biased pairs
N=1227
Bias =−4.828
BnC
Biased pairs
N=2746
Bias =4.872
count
Bias
Bna Biased
BnC Biased
Nonbiased
Log2(Expr_BnC/Expr_BnA)

**Figure S5.**



**Figures S1-S5** Distribution of homoeolog bias in the parent and three generations of line EL 200, EL 300, EL 400, EL 600, and EL 1100, red regions indicate BnC biased homoeologs with log2 expression foldchange greater than 3.5 and blue regions indicate BnA biased homoeologs with log2 expression foldchange less than -3.5.

# Figure S6

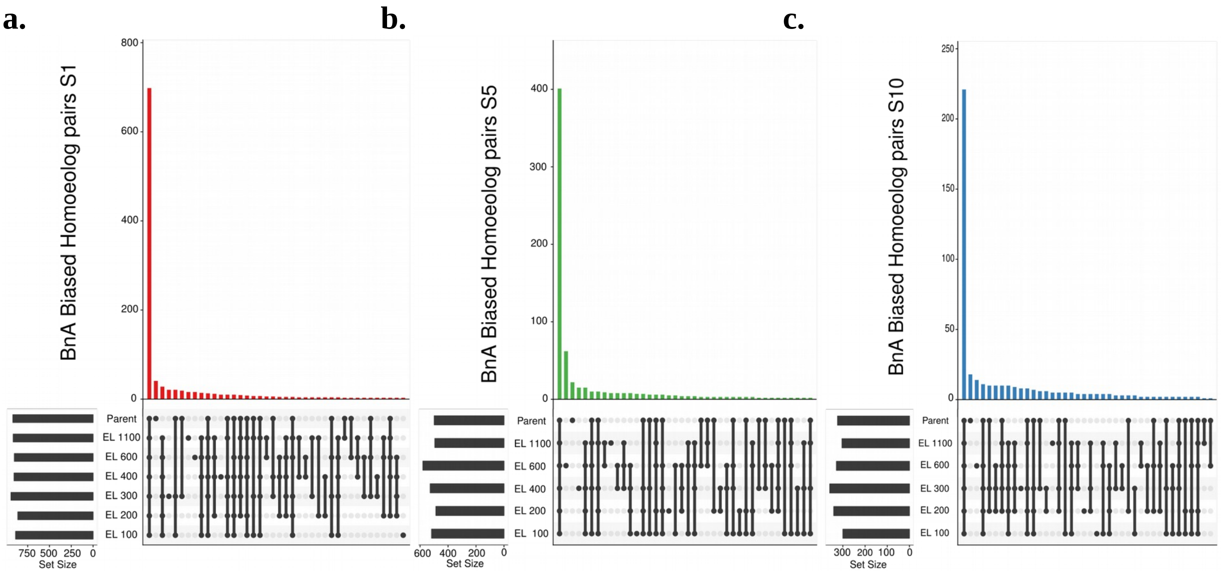


**Figure S6** Common shared biased Homoeolog pairs: Upset plot of how biased homoeologs pairs for BnA biased (**a-c**) are shared among all six lines for the three sampled generations. This analysis was restricted only to homoeolog pairs in 2:2 balance in all 6 lines.

## Figure S7

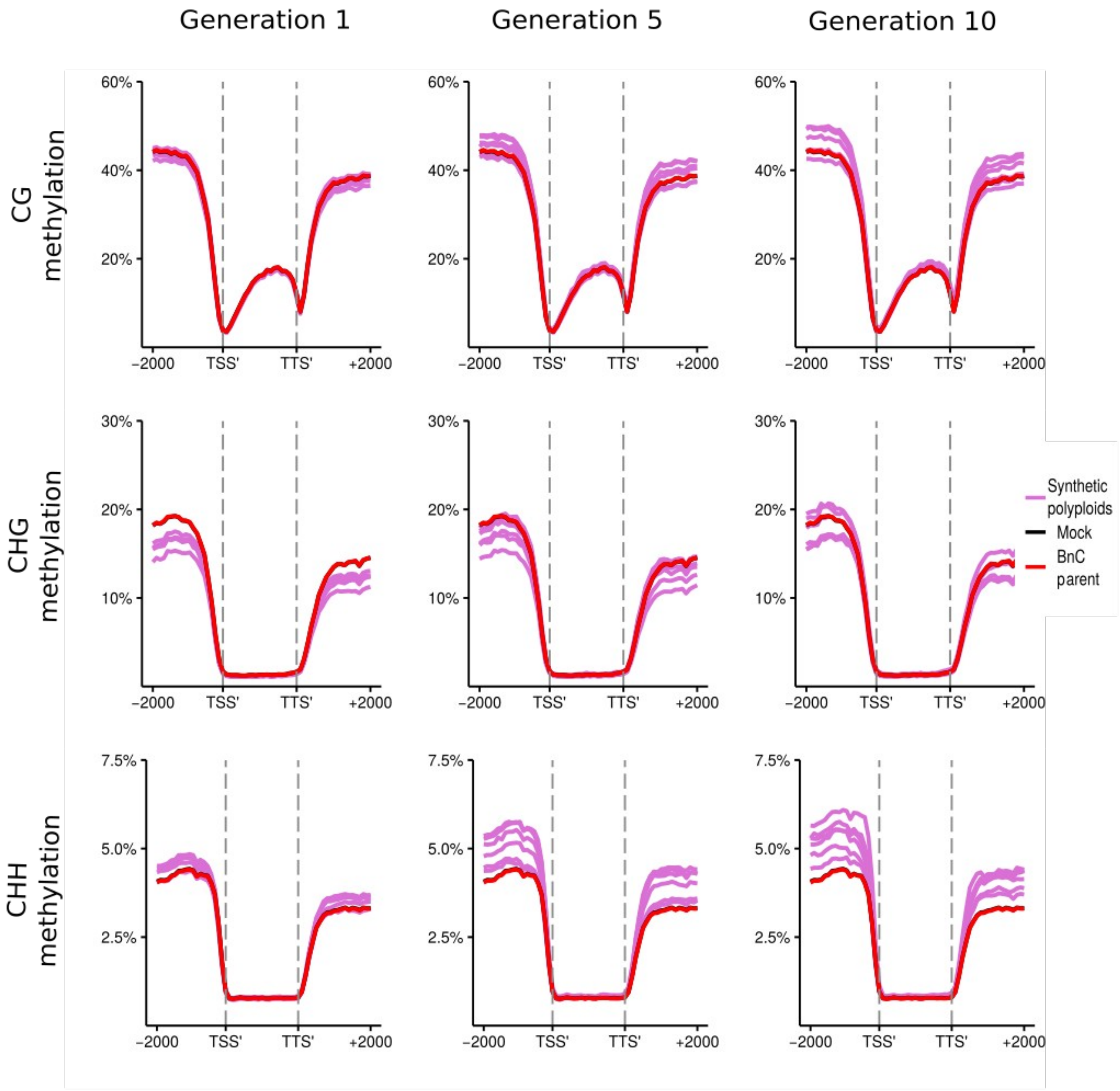


**Figure S7: DNA Methylation of BnC syntenic genes.**

Metaplots of CG, CHG, and CHH mean weighted methylation of syntenic on the BnC subgenome for generations one, five, and ten assessed using Bisulfite-seq. The six resynthesized lines are shown in purple, the *Brassica oleracea* progenitor is shown in red and *in silico* mock polyploid in black as a visual control for cross mapping errors. Methylation levels are shown for the transcription start site (TSS'), gene body, transcription termination site (TTS') and 2kb up- and downstream of the TSS and TTS**.**

## Figure S8

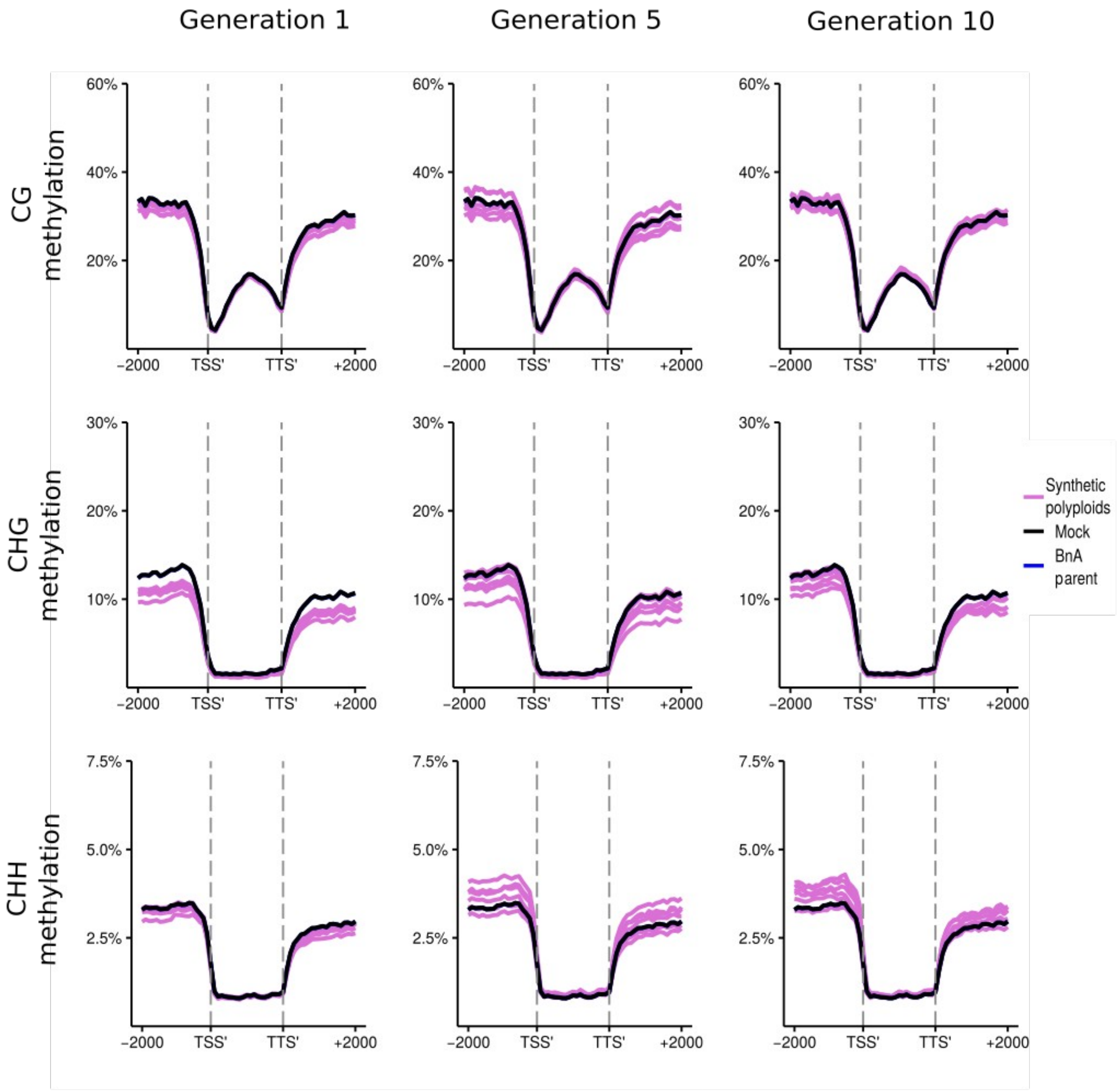


**Figure S8: DNA Methylation of BnA Syntenic Genes.**

Metaplots of CG, CHG, and CHH mean weighted methylation of all syntenic genes on the BnA subgenome for generations one, five, and ten assessed using Bisulfite-seq. The six resynthesized lines are shown in purple, the *Brassica rapa* progenitor is shown in blue and *in silico* mock polyploid in black as a visual control for cross mapping errors. Methylation levels are shown for the transcription start site (TSS’), gene body, transcription termination site (TTS’) and 2kb up- and downstream of the TSS and TTS.

## Figure S9

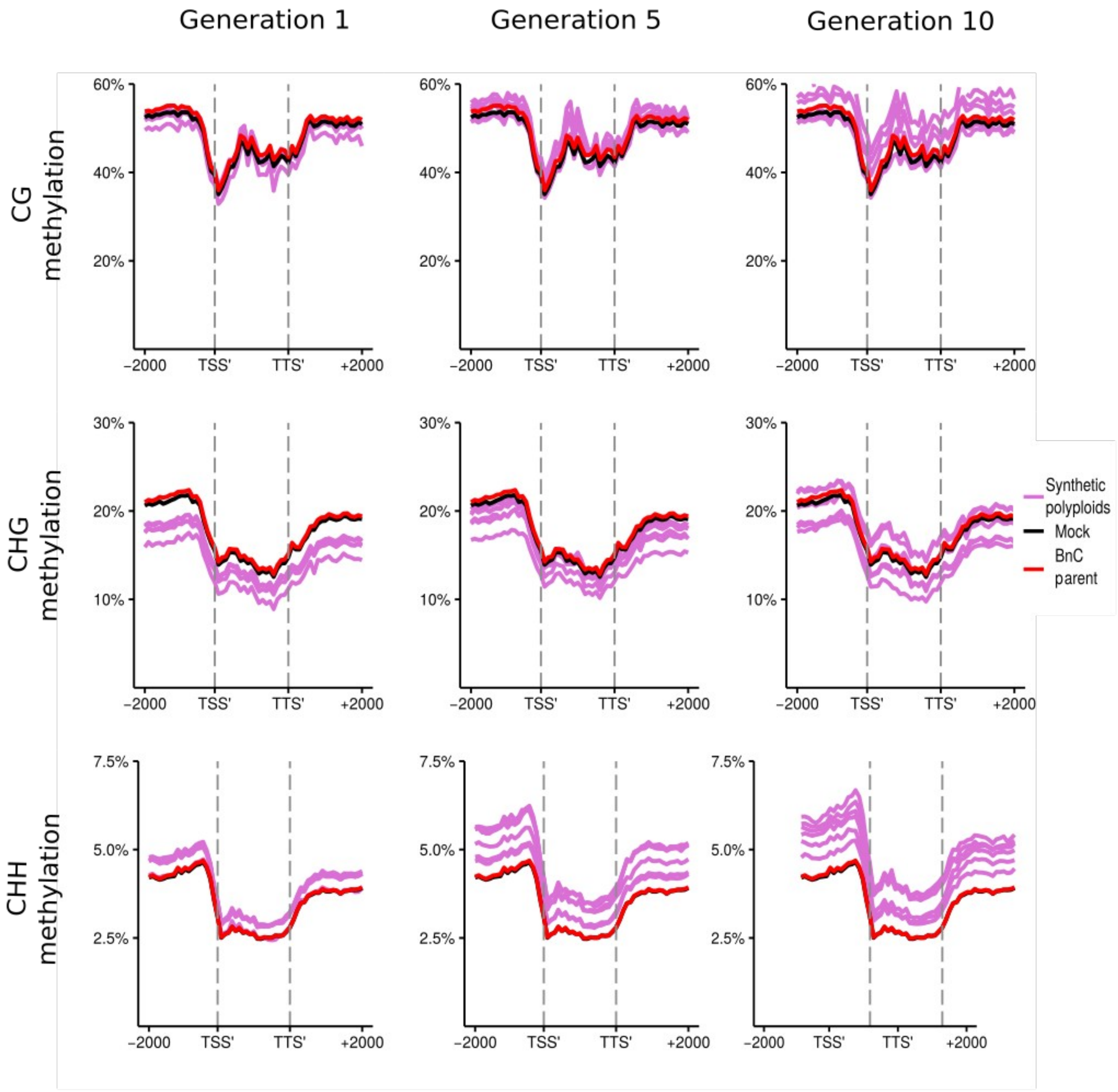


**Figure S9: DNA Methylation of BnC Non-Syntenic Genes.**

Metaplots of CG, CHG, and CHH mean weighted methylation of non-syntenic genes on the BnC subgenome for generations one, five, and ten assessed using Bisulfite-seq. The six resynthesized lines are shown in purple, the *Brassica oleracea* progenitor is shown in red and *in silico* mock polyploid in black as a visual control for cross mapping errors. Methylation levels are shown for the transcription start site (TSS'), gene body, transcription termination site (TTS') and 2kb up- and downstream of the TSS and TTS.

**Figure S10**

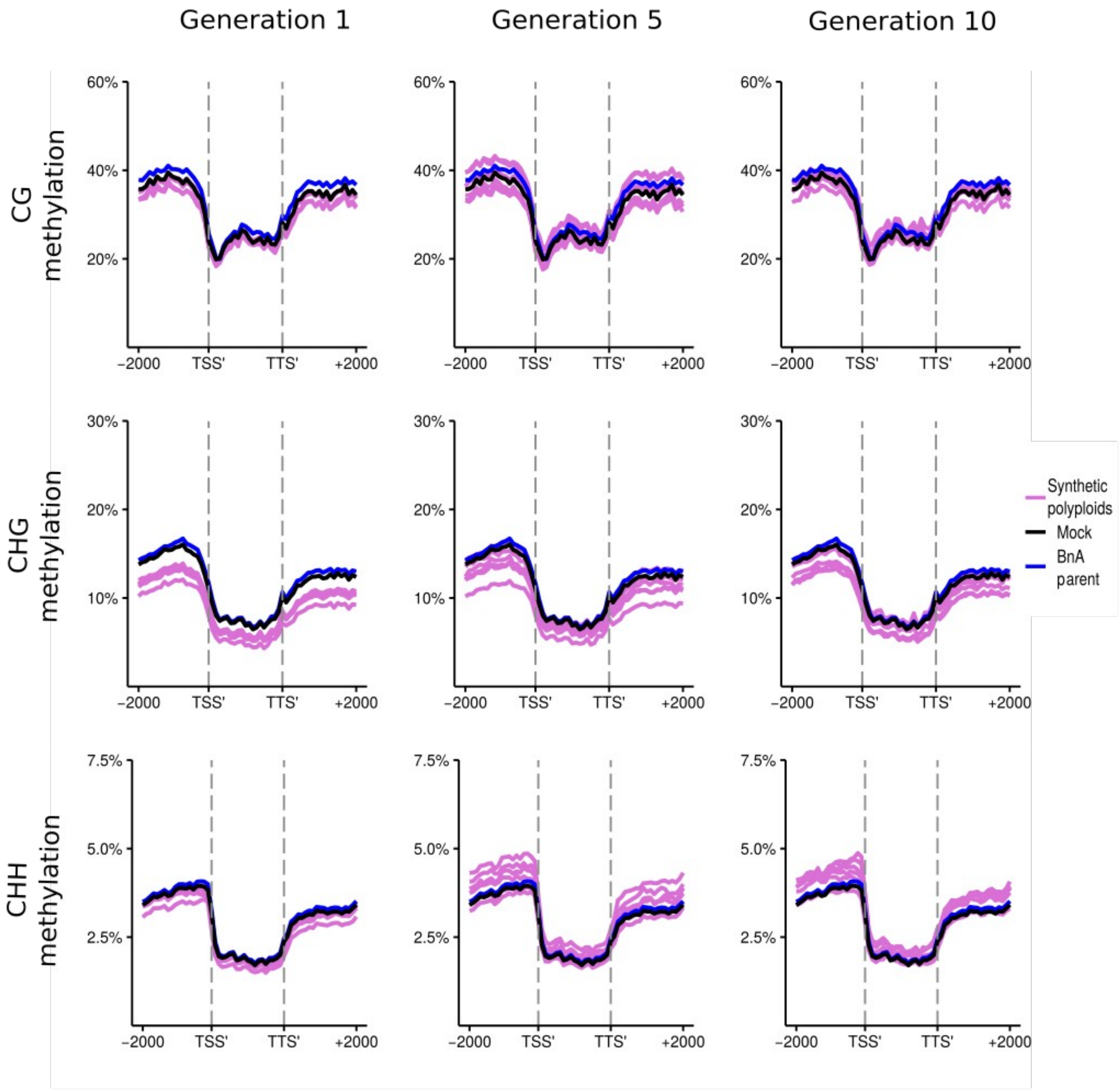


**Figure S10: DNA Methylation of BnA Non-Syntenic Genes.**
Metaplots of CG, CHG, and CHH mean weighted methylation of non-syntenic genes on the BnA subgenome for generations one, five, and ten assessed using Bisulfite-seq. The six resynthesized lines are shown in purple, the *Brassica rapa* progenitor is shown in blue and *in silico* mock polyploid in black as a visual control for cross mapping errors. Methylation levels are shown for the transcription start site (TSS'), gene body, transcription termination site (TTS') and 2kb up- and downstream of the TSS and TTS**.**

**Table S1:**

Homoeolog Expression Bias Chi Squared table

| Line | BnC. Observed | BnC. Expected | BnA. Observed | BnA. Expected | Chi.Squared | P.value |
|---|---|---|---|---|---|---|
| 100S1 | 3051 | 2182 | 1313 | 2182 | 692.17 | 1.51e-152 |
| 100S5 | 2411 | 1746.5 | 1082 | 1746.5 | 505.65 | 5.60e-112 |
| 100S10 | 2407 | 1666.5 | 926 | 1666.5 | 658.07 | 3.92e-145 |
| 200S1 | 3138 | 2232.5 | 1327 | 2232.5 | 734.54 | 9.23e-162 |
| 200S5 | 2562 | 1903 | 1244 | 1903 | 456.42 | 2.90e-101 |
| 200S10 | 2302 | 1811 | 1320 | 1811 | 266.24 | 7.49e-60 |
| 300S1 | 3085 | 2281.5 | 1478 | 2281.5 | 565.95 | 4.26e-125 |
| 300S10 | 2355 | 1758.5 | 1162 | 1758.5 | 404.68 | 5.28e-90 |
| 400S1 | 3342 | 2444.5 | 1547 | 2444.5 | 659.04 | 2.42e-145 |
| 400S5 | 2873 | 2184 | 1495 | 2184 | 434.73 | 1.52e-96 |
| 600S1 | 3501 | 2589.5 | 1678 | 2589.5 | 641.69 | 1.43e-141 |
| 600S5 | 2440 | 2022.5 | 1605 | 2022.5 | 172.37 | 2.25e-39 |
| 600S10 | 2746 | 1986.5 | 1227 | 1986.5 | 580.76 | 2.56e-128 |
| 1100S1 | 3060 | 2235 | 1410 | 2235 | 609.06 | 1.79e-134 |
| 1100S5 | 2683 | 1918.5 | 1154 | 1918.5 | 609.29 | 1.60e-134 |
| 1100S10 | 2955 | 2014 | 1073 | 2014 | 879.33 | 3.06e-193 |
| Parent | 3688 | 2742 | 1796 | 2742 | 652.75 | 5.65e-144 |

**Table S2**

Homoeolog Expression Bias vs Parent Chi Squared table

| Line | BnC Observed | BnC Expected | BnA Observed | BnA Expected | Chi.Squared | P.value |
|---|---|---|---|---|---|---|
| 100S1 | 3051 | 2934.798 | 1313 | 1429.202 | 14.049 | 0.0002 |
| 100S5 | 2411 | 2349.049 | 1082 | 1143.951 | 4.9888 | 0.03 |
| 100S10 | 2407 | 2241.448 | 926 | 1091.552 | 37.336 | 9.94e-10 |
| 200S1 | 3138 | 3002.721 | 1327 | 1462.279 | 18.61 | 1.60e-05 |
| 200S5 | 2562 | 2559.542 | 1244 | 1246.458 | 0.0072085 | 0.9323 |
| 200S10 | 2302 | 2435.802 | 1320 | 1186.198 | 22.443 | 2.17e-06 |
| 300S1 | 3085 | 3068.626 | 1478 | 1494.374 | 0.26679 | 0.6055 |
| 300S10 | 2355 | 2365.189 | 1162 | 1151.811 | 0.13402 | 0.7143 |
| 400S1 | 3342 | 3287.861 | 1547 | 1601.139 | 2.722 | 0.09897 |
| 400S5 | 2873 | 2937.488 | 1495 | 1430.512 | 4.3229 | 0.0376 |
| 600S1 | 3501 | 3482.887 | 1678 | 1696.113 | 0.28763 | 0.5917 |
| 600S5 | 2440 | 2720.27 | 1605 | 1324.73 | 88.172 | < 2.2e-16 |
| 600S10 | 2746 | 2671.85 | 1227 | 1301.15 | 6.2836 | 0.01219 |
| 1100S1 | 3060 | 3006.083 | 1410 | 1463.917 | 2.9528 | 0.08573 |
| 1100S5 | 2683 | 2580.389 | 1154 | 1256.611 | 12.459 | 0.00042 |
| 1100S10 | 2955 | 2708.837 | 1073 | 1319.163 | 68.305 | < 2.2e-16 |